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ANTI ULCER ACTIVITY OF ETHANOLIC EXTRACT OF DIOSPYROS PANICULATA

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ABSTRACT

This study was conducted to evaluate the anti ulcer activity of the ethanolic extract of Diospyros Paniculata(bark). The study was performed by doing the Indomethacin induced ulcer in rats and ethanol induced ulcers in rat. The ulcer index is calculated and also the Percentage protection. Results obtained in both of these models show significant Anti ulcer activity with $P < 0.01$. Findings obtained indicate that the Diospyros Paniculata is having significant anti ulcer activity.

KEYWORDS: Diospyros Paniculata, Anti ulcer, In vivo, Indomethacin, ethanol.

INTRODUCTION

Plants are important and basic of preventive and curative health cares system since immemorial. Disease is as old as mankind and use of indigenous herbal medicine is a very ancient art and an integral part of treatment.[1] Traditional medicinal herbs have served as a potential source of alternative medicine and different healthcare products. Knowledge of herbal medicines has derived from rich traditions of ancient civilizations and scientific heritage. From ancient time Indian, Chinese, Egyptian, Greek, Roman and Syrian medicinal system documented the use of different plant based medicine for different diseases.[2] Folk medicines and their use against diseases in different cultures is a vast traditional knowledge; which is based on the necessities, instinct, observation, trial and error and long experience of ancient/tribal people.[3] Indigenous or herbal medicines confer considerable economic benefits to most rural and poor people. WHO noted that about 25% of modern medicines are descended from plants sources used traditionally and research on traditional medicinal herbal plant leads discovery of 75% of herbal drugs.[4] aHerbal medicines are at a stage of come back which is having much safety as compared to synthetic medicines having unwanted side effects.

Peptic ulcers are thought to develop because of an imbalance between aggressive factors and protective factors.[5] The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs.[6] In spite of the rapidly changing concept of gastric ulcer management from conventional vagotomy, prostaglandin analogs, H2 receptor antagonists and antacids to proton pump inhibitors, gastrointestinal toxicity remains an impediment to their application in clinical practice. Specifically, gastrointestinal toxicity of non-steroidal anti-inflammatory drugs (NSAIDs) origin maybe as high as 4–8% per year and the complications are even higher for those with additional risk factors such as prior history of ulcer disease.[7] The main aim of the treatment is pain management, heal ulcers and to prevent the re occurrences.

Diospyros is a large genus of trees or shrubs, belonging to family Ebanaceae, which are widely distributed in both the hemispheres. About 41 species are indigenous to India, mostly in evergreen forest of Deccan, Assam and Bengal; a few have been reported in North India.[8] Its bark is bitter, astringent and febrifuge. The unripe fruit is a more powerful astringent.[9] In Indian system of traditional medicines like Ayurveda and Unani, various *Diospyros* species are used medicinally to cure fever, diabetes, snake bite, diarrhea, biliousness, ulcer etc.[10] But till now there has been no scientific work has been reported proving the anti ulcer effect of diospyros paniculata. Current study is performed to evaluate the anti ulcer potential of diospyros paniculata. The preliminary photochemical screening of diospyros paniculata indicate the presence of essential oil, saponins, terpenoids, flavanoids and alkaloids.[11,12]

MATERIALS AND METHODS

PLANT COLLECTION AND AUTHENTICATION

Diospyros Paniculata was collected from Gootrical Forest range, sabarimala, Pathanamthitta district, kerala in October 20, 2015 and it was authenticated by Mr. M V Krishna raj M.Sc., B.Ed., Ph.D Assistant Professor, Department of Botany, Baselius College Kottayam.

PREPERATION OF THE ETHANOLIC EXTRACT OF BARK OF DIOSPYROS PANICULATA

Fresh bark of Diospyros Paniculata was collected and washed thoroughly with distilled water and dried in open air at shade. Later the dried bark were chopped into small pieces and the material were properly packed and kept in soxhlet extractor and is made to undergo successive soxhlet extraction using ethanol as solvent. After 48hrs the extract was collected and it is air dried to remove the solvent. The extract collected is properly packed and kept for further studies.

ACUTE TOXICITY STUDIES (OECD Guidelines 423)[13]

The preliminary pharmacological studies were conducted to assess the acute pharmacological effects of ethanolic extract of Diospyros paniculata bark. The acute toxicity study was carried out in female Swiss albino mice under the OECD Guidelines 423. The animals were fastened overnight and the extracts of the Diospyros Paniculata were suspended in 0.5% CMC and administered starting at 2000mg/kg, food was withheld for next 3-4 hours. The animals were observed continuously for changes in physical appearance, the gross behavioral changes like signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma was closely monitored for every 30 minute for next 3 hours and till 24 hours. And the animals were kept at close observation for 14 days for any signs of long term toxic effects.

EXPERIMENTAL ANIMALS

Healthy strains of Swiss albino mice were use for performing acute toxicity studies and adult wistar Albino rats were used for doing in vivo pharmacological evaluation, which is collected from Animal house, Department of Pharmacology, DPS, MGU, RIMSR, Kottayam(IAEC No.1702/PO/C/CPCSEA).

IN VIVO MODELS

INDOMETHACIN INDUCED ULCER MODEL[14,15,16]

NSAIDS category drugs can cause gastric lesions in the humans and also in the experimental animals by inhibiting COX.

All the animals were fasted 36 hours before administration of Indomethacin with free access to water but water is also withdrawn 1 hour before experiment. Animals were divided into five groups 1,2,3,4,5 each group having six animals each. The dose of the test was calculated based on Acute toxicity studies.

Group 1 - Negative control (Receive only CMC only)
Group 2 – Positive control (Receive Indomethacin 20mg/kg only)
Group 3 – Standard group (Receive Omeprazole 20mg/kg & Indomethacin 20 mg/kg only)
Group 4 - Test group 1 (Extract 200mg/kg & Indomethacin 20 mg/kg only)
Group 5 – Test group 2 (Extract 400mg/kg & Indomethacin 20 mg/kg only).

All the animals received the drug as per the procedure later after 30 minutes the inducing agent Indomethacin is administered. After 8 hours of drug treatment the animals were euthanized and stomach was removed opened along the greater curvature and ulcer counted and the percentage protection also calculated later the stomach is taken for further histopathological examination.

Ulcer index calculated by noting the number of ulcers per animal and severity scored by observing the ulcers microscopically with the help of 10x lens and scoring is done as below.

- 0 - Normal stomach
- 0.5 - Red coloration
- 1 - Spot ulcers
- 1.5 - Hemorrhagic streaks
- 2 - Ulcer > 3 mm but ‹ 5 mm
- 3 - Ulcers > 5 mm

Calculation of ulcer Index

UI = UN + US + UP x 10-1
UI = Ulcer Index
UN = Average of number of ulcer per animal
US = Average of severity score
UP = Percentage of animal with ulcer
And percentage protection was observed by using the formula:

$$\%\text{protection} = \frac{\text{Ulcer index control} - \text{Ulcer index test}}{\text{Ulcer index control}}$$

ETHANOL INDUCED ULCER MODEL[17,18]

Alcohol causes secretion of gastric juice and decrease mucosal resistance due to which protein content of gastric juice is significantly increased by ethanol. This could be leakage because of plasma protein in the gastric juice with weakening of mucosal resistance barrier of gastric mucosa, this leading to peptic ulcer.

All the animals were fasted for 24 hours before starting the experiment. Animals were divided into five groups 1,2,3,4,5 each group having 6 animals each.

Group 1 – Negative control (Receive CMC only)
Group 2 – Positive control (Receive 1ml/kg of 80% ethanol)
Group 3 – Standard group (Received omeprazole 20mg/kg & 1ml/kg of 80% ethanol)
Group 4 - Test group 1 (Extract 200mg/kg & 1ml/kg of 80% ethanol)
Group 5 – Test group 2 (Extract 400mg/kg & 1ml/kg of 80% ethanol)
Group 1 received CMC only, Group 2 received 1ml/kg 80% ethanol, Group 3 received omeprazole 20mg/kg, Group 4 & 5 received 200 and 400 mg/kg of the Extract respectively. After one hour all the animals received 1ml/kg of ethanol orally. After 8 hours the rats were sacrificed by cervical dislocation and the stomachs were

isolated, washed gently with formol saline cut open along the greater curvature. The stomachs were fixed in 10% formalin and craters observed and ulcer score calculated, percentage protection later the stomach is taken for further histopathological examination.

Ulcer index calculated by noting the number of ulcers per animal and severity scored by observing the ulcers microscopically with the help of 10x lens and scoring is done as below.

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$$\%\text{protection} = \frac{\text{Ulcer index control} - \text{Ulcer index test}}{\text{Ulcer index control}}$$

HISTOPATHOLOGICAL STUDIES
A portion of the region in the rat stomach was dissected out and fixed in 10% formalin solution for histopathological observations. After fixation the tissues were embedded in paraffin, solid sections were cut at 5µm and stained with haemotoxylin and eosin. The sections were examined with the help of a light microscope and photomicroscope were taken and scored.

RESULTS
1. EXTRACTION
The ethanolic extract of diospyros paniculata (Bark) is prepared.

2. ACUTE TOXICITY STUDIES
Acute toxicity studies of the Ethanolic extract of Diospyros paniculata is performed according to the Guidelines 423 and it is found that the extract is safe up to 2000mg/kg body weight by oral route. Animal did not show any signs of toxicity so that the dose of 200mg/kg and 400mg/kg is selected for further study.

3. IN VIVO METHODS
3.1 INDOMETHACIN INDUCED ULCER MODEL
The effect of indomethacin induced ulcer model in ethanolic extract of diopyros paniculata is shown in Table 1. In this model the ulcer index of toxic control group is found to be 10.521 ± 0.021 and ulcer index of standard group found to be 1.713 ± 0.766, ulcer index of test 200mg/kg was found to be 3.366 ± 1.065 and test 400mg/kg is 2.2 ± 0.700. It is found that the extract is showing significant Anti ulcer activity with a Percentage protection of 83% in standard group, 68% in test 200mg/kg and 79% in test 400mg/kg.

Table 1.Effect of Ethanolic extract of Diospyros Paniculata

SL NO:	GROUP	ULCER INDEX	PERCENTAGE PROTECTION	F value
1	Negative control	0	0	
2	Toxic control			
3	Standard group(omeprazole 20mg/kg&indomethacin)	10.521±0.021	0	F value = (3,20)=30.45
4	Test 1, 200mg/kg(Extract 200mg/kg& indomethacin)	1.713 ± 0.766** 3.366±1.065**	83% 68%	
5	Test 2, 400mg/kg(Extract 400mg/kg& indomethacin)	2.2±0.700**	79%	

The values were expressed as mean±SEM, n=6 for each group and significance between various groups at P<0.01** by one way ANOVA followed by Dunnets t-test.

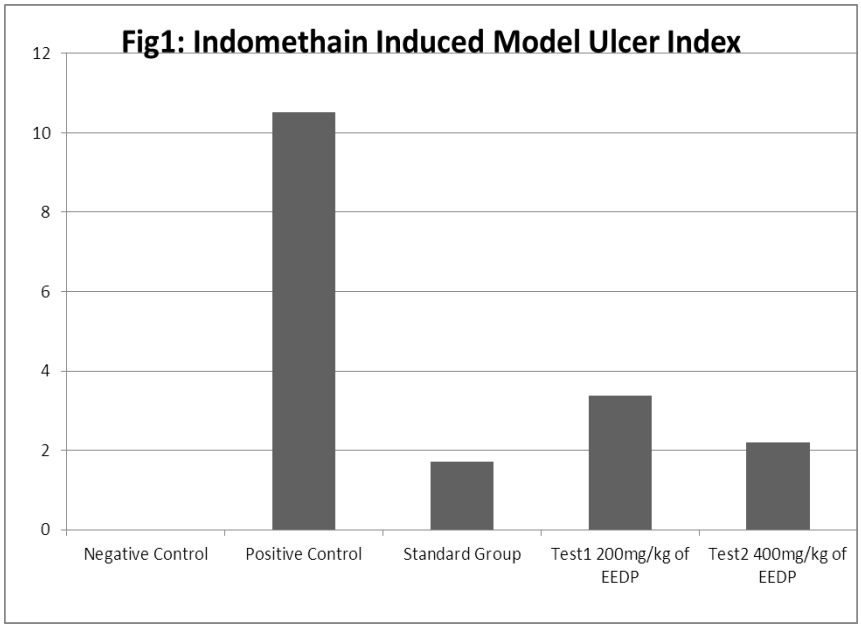
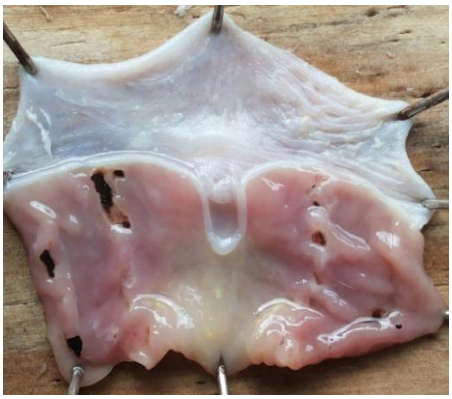
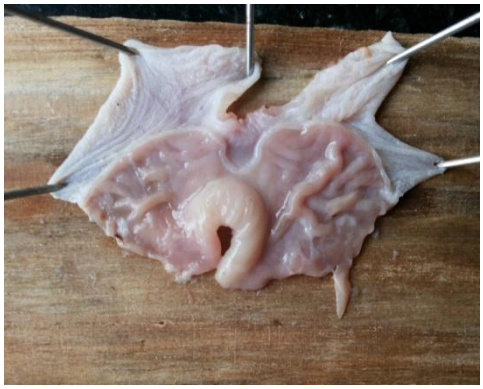


Figure: 1 Effect of EEDP in indomethacin induced ulcer

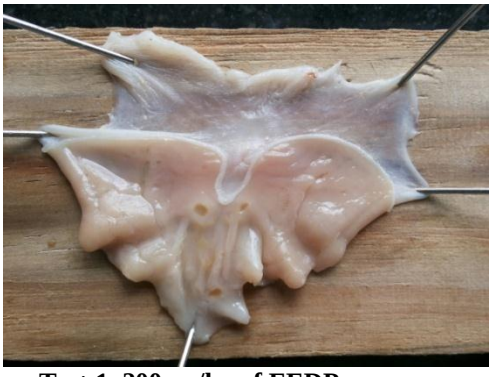
Negative control



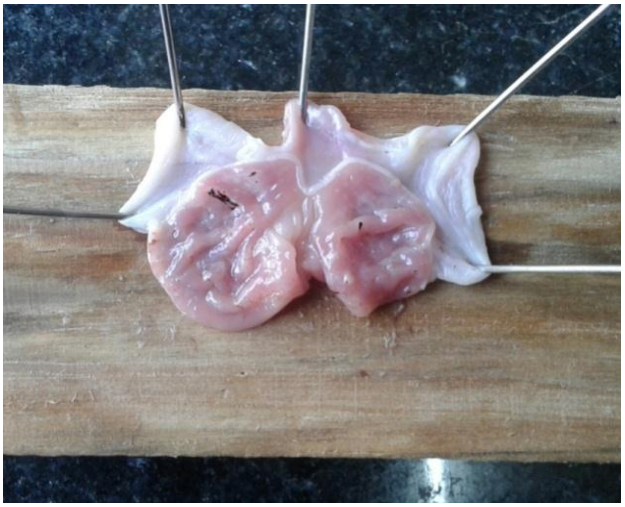
Positive control



Standard group



Test 1, 200mg/kg of EEDP



Test 2, 400mg/kg of EEDP

3.2 ETHANOL INDUCED ULCER MODEL

Figure 2: Macroscopical view of indomethacin induced ulcer model in rat

The effect of ethanol induced ulcer model in ethanolic extract of diopyros paniculata is shown in Table 2. In this model the ulcer index of toxic control group is found to be 10.40±0.0324 and ulcer index of standard group is found to be 1.668±0.746, ulcer index of test 1, 200mg/kg is 3.37±1.066 and the ulcer index of test 2, 400mg/kg is found to be 2.226±0.704. It is found that the extract is showing significant Anti ulcer activity with a Percentage protection of 82% in standard group, 67% in test 200mg/kg and 76% in test 400mg/kg.

Table 2. Effect of Ethanolic extract of Diospyros Paniculata

SL NO:	GROUP	ULCER INDEX	PERCENTAGE PROTECTION	F value
1	Negative control	0	0	
2	Toxic control	10.40±0.0324	0	
3	Standard group(omeprazole 20mg/kg & indomethacin)	1.668 ± 0.746**	82%	F value = (3,20)=29.967
4	Test 1, 200mg/kg(Extract 200mg/kg& indomethacin)	3.37±1.066**	67%	
5	Test 2 400mg/kg(Extract 400mg/kg & indomethacin)	2.226±0.704**	76%	

The values were expressed as mean±SEM, n=6 for each group and significance between various groups at $P<0.01^{**}$ by one way ANOVA followed by Dunnets t-test.

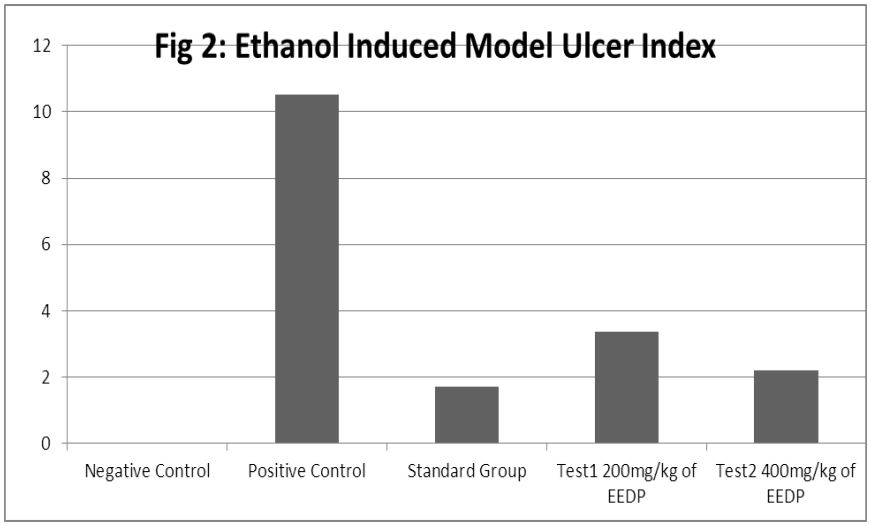
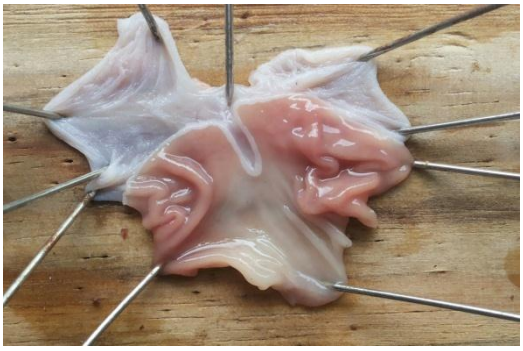
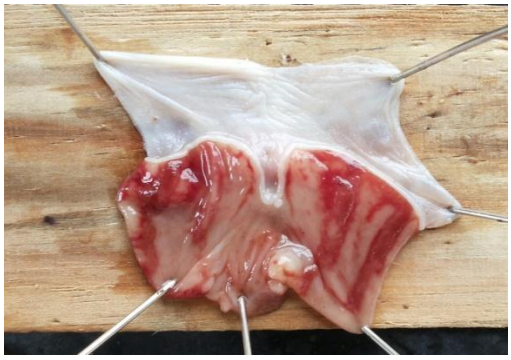


Figure 2: Effect of EEDP in ethanol induced ulcer model



Negative control



Toxic control

Standard control

Test 1, 200mg/kg of EEDP

Test 2, 400mg/kg of EEDP

Figure 2: Macroscopical view of ethanol induced ulcer models in rat

DISCUSSION

In indomethacin induced ulcer model ulcer occur due to the inhibition of the prostaglandins which are having a protective effect on the mucosa. In case of ethanol induced ulcer model the ulcerogenic effect due to the increased secretion of the gastric juice and also due to the decreased mucosal resistance. In both of these models the ethanolic extract of diospyros paniculata is showing significant anti ulcer activity and better gastric protection.

The preliminary photochemical analysis indicate that it composed of chemical constituents like flavanoids, alkaloids, saponins etc which are having significant anti ulcer activity. so that these chemical constituents may be responsible for the anti ulcer activity of the diospyros paniculata. Even though detailed study of the chemical components of the bark is required for confirming the anti ulcer effect of the plant.

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